DIFFERENTIAL EXPRESSION OF DNA DOUBLE-STRAND BREAK-REPAIR PROTEINS IN HUMAN BREAST CELLS

Mangala Tawde, Paul Freimuth, and Carl W. Anderson

Biology Department, Brookhaven National Laboratory,
Upton, NY 11973

cwa@bnl.gov

DNA double-strand break (DSB) repair is critical for cell survival and for preventing genomic rearrangements that lead to cancer. In mammalian cells, non-homologous end joining (NHEJ), which requires the DNA-dependent protein kinase complex (DNA-PKcs, Ku70, Ku80), XRCC4 and Ligase IV, is the major pathway for DSB repair. In vertebrates, DNA-PKcs, the catalytic component of DNA-PK, is an essential pathway component.

Recent studies of mice found lower levels of DNA-PKcs expression in breast tissue compared to other mouse tissues. Furthermore, BALB/c mice, which are 20 times more susceptible to low dose radiation-induced breast cancer than the most other mouse strains (e.g., C57Bl/6), expressed less DNA-PKcs protein than radiation-resistant strains. Thus, breast cancer susceptibility has been associated with DNA DSB repair capacity, radiosensitivity and DNA-PKcs expression. Cell lines from more than 40 % of cancer patients exhibit increased genomic instability. However no correlation has yet been made between radiosensitivity in cancer patients and the expression and activities of DNA repair proteins in fibroblast cell lines derived from these patients.

Our earlier studies showed that DNA-PKcs was differentially expressed in normal and malignant human tissues, with highest levels in neurons and glial cells and lowest in liver and resting breast tissues. All tissues showed similar mRNA content for the protein suggesting the poor expression of DNA-PK could result from post-transcriptional mechanisms rather than transcriptional regulation. When the above observations were recapitulated by studying selected human tissue sections by immunohistochemistry (IHC) for expression of the NHEJ proteins, a striking cell-to-cell specificity was observed. Epithelial cells showed strong staining while stromal cells exhibited very little NHEJ protein expression. To model these *in-vivo* findings *in-vitro*, a cell line of breast stromal origin (Hs 574.T; ATCC CRL-7345) is being characterized for expression of all NHEJ repair proteins by immunohistochemistry, immunofluorescence, and immunoblotting using prepared and commercial antisera specific for each of the NHEJ proteins. Preliminary studies have shown lower activity but comparable expression of DNA-PKcs and the Ku subunits in this cell line. The XRCC4/Lig IV activity, and the genome instability of these cells also is under investigation. Cell lines derived from breast tumors are being examined for polymorphisms in the coding sequences of the NHEJ genes.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0168 supported this work.

IMMATURE RAT MAMMARY EPITHELIAL CELLS ARE MORE SUSCEPTIBLE THAN MATURE CELLS TO THE CARCINOGENIC AND MUTAGENIC EFFECTS OF N-NITROSO-N-METHYLUREA

Jennifer L. Ariazi, Jill D. Haag, Mary J. Lindstrom, and Michael N. Gould

University of Wisconsin - Madison

jariazi@students.wisc.edu

Although studies indicate that the immature human breast is more susceptible than the mature breast to the carcinogenic effects of ionizing radiation, analogous data on chemical breast carcinogens are lacking due to the focus of epidemiology on adult exposures. The work presented here was undertaken to explore the age-differential susceptibility of the breast using the Fischer 344 rat model. Immature (3-week-old) and mature (8-week-old) rats were treated with dimethylbenzanthracene (DMBA) or N-nitroso-N-methylurea (NMU) and followed by weekly palpation. As expected, carcinoma incidences and multiplicities were greater in mature than immature DMBA-treated rats. However, in NMU-treated rats, carcinoma incidences and multiplicities were significantly greater in immature than mature rats. These results indicate a carcinogen-specific elevated susceptibility of immature rat mammary epithelial cells (RMECs). To determine whether this age-differential susceptibility to NMU extends to mutagenesis, immature and mature lacI transgenic (Big Blue) rats were treated with NMU and sacrificed after various expression periods. At all timepoints, immature RMECs exhibited significantly higher mutant frequencies than mature RMECs, indicating that immature RMECs are more susceptible also to the mutagenic effects of NMU. To explore earlier events, single cell gel electrophoresis (comet) assays were performed on immature and mature RMECs exposed to NMU in short-term culture. No age-differential effects were observed immediately following treatment. However, beginning two hours after treatment, the tail moments of immature, but not mature, RMECs increased. Apoptosis was not responsible. Inhibition of methylguanine methyltransferase (MGMT) by benzylguanine pretreatment did not affect the immature RMECs but caused the mature RMECs to recapitulate the immature response. Preliminary MGMT activity assays support the hypothesis that immature RMECs are deficient in MGMT activity. Immature RMECs, which must then use more error-prone repair, harbor more mutations, elevating their cancer risk. These findings suggest that the search for environmental -- preventable -- causes of breast cancer should focus on exposures of the immature breast and its potential DNA repair deficiencies.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0503 supported this work.

EXPRESSION OF A DEFECTIVE REPAIR GENE, DNA POLYMERASE β, IN MAMMARY GLANDS OF TRANSGENIC MICE

Sipra Banerjee and Nandan Bhattacharyya

Department of Cancer Biology, Lerner Research Institute,
Cleveland Clinic Foundation, 9500 Euclid Avenue,
Cleveland, OH 44195

banerjs@ccf.org

DNA polymeraseβ (polβ) is a major contributor to gap filling synthesis of damaged DNA in base excision repair mechanism. We provided the first evidence for a high prevalence of a specific 87 bp deletion encoding amino acid residues 208-236 in cDNA of polβ in human breast adenocarcinomas. A wild-type and a truncated polβ (polβΔ) proteins are also expressed in breast tumor cell extracts. A significantly reduced level in gap filling and DNA binding activities is identified in cells expressing the polβΔ protein. These cells are found to be hypersensitive to MNNG, a DNA alkylating agent. More importantly, the polβΔ acts as a dominant mutant. To elucidate whether polβΔ has a contributory role *in vivo* in increased susceptibility to mammary carcinogenesis, we have initiated to establish transgenic mouse model expressing the polβΔ. A polβΔ208-236 transgene construct under the control of a strong mammalian mammary epithelial cell specific promoter, whey acidic protein (WAP) (kindly provided by Dr. J. Rosen) was made. The 2.9 kb construct was microinjected into pronuclei of B6CBA mice by our transgenic core. DNA isolated from tail-clips of 2 weeks old pups were analysed by Southern blot hybridized with a 900 bp polβ probe. Six positive founders were identified. These animals were mated with wild-type C57bl mice. Positive Southern results identified animals with the transgene suggesting polβΔ F1 animals have been established. To evaluate whether the polβ transgene is expressed in mammary glands, pregnant F1 mice were used. The reason behind use of pregnant animals is WAP is expressed when prolactin and progesterone are secreted in serum at a high level. Cell extracts were made from mammary glands of F1 mice one week after delivery of littermates. Expression of polβ protein in extracts was determined by Western blot using an anti-polβ antibody. Expression of a 36 kDa polβΔ protein in mammary glands along with an endogenous WT 39 kDa polβ protein were identified in F1 mice. Only a WT polβ protein was expressed in extracts of mammary glands of nontransgenic mice having similar genetic background. We are continuing breeding of F1 mice to expand colonies of transgenic animals. To test whether transgenic animals are more susceptible to breast cancer than wild-type animals, we have initiated a pilot study by injecting one-week-old pregnant mice intraperitoneally with 30mg/kg body weight of methylnitrosourea. Treatments were continued for five weeks. Animals are closely examined every third day by palpating mammary glands. The weights of the animals are recorded. A control group of nontransgenic animals of same age received the carcinogen for identical dose and time. This group will provide estimates of the tumor induction in normal animal.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9131 supported this work.

A CONDITIONAL ALLELE OF BRCA1

Blase Billack, Terri Worley, Johan Vallon-Christersson, Ake Borg, and Alvaro N. A. Monteiro

Laboratory of Molecular Oncology, Strang Cancer Prevention Center, and Department of Cell and Developmental Biology, Weill Medical College of Cornell University, New York, NY 10021

billacb@rockefeller.edu

The majority of cases of hereditary breast and ovarian cancer in the United States are linked to mutations in the breast and ovarian cancer susceptibility gene BRCA1. Several physiological roles of BRCA1 have been described which include control of cell cycle, activation of transcription and modulation of DNA damage response. The mechanism by which BRCA1 mediates these processes remains unclear. We have identified and characterized a naturally-occurring cancer-predisposing allele of BRCA1 with a missense mutation that displays temperature-sensitive activity. When the carboxy-terminus of BRCA1 carrying the mutation protein was fused to a GAL4 DNA-binding domain and expressed in yeast, it was able to activate transcription of a GAL4-responsive reporter gene in a temperature-sensitive manner. Interestingly, this mutant was also able to activate transcription in human embryonic kidney cells in a temperature-dependent manner. We further observed that this mutant is also temperature-sensitive in HCC1937, human cells lacking functional BRCA1. Next, we expressed this mutant in a full length context in HCC1937 and found that both subcellular localization and phosphorylation status are similar to wild type at both permissive and non-permissive temperature. Preliminary results of transient transfections suggest that the mutant is able to restore radioresistance to HCC1937 only at the permissive temperature. We have begun to use this mutant to analyze the role of BRCA1 in cell cycle control and DNA damage response. Our results suggest that this conditional mutant can be used as a tool to dissect the role of BRCA1 in breast and ovarian cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0403 supported this work.

INHIBITION OF THE DNA REPAIR PROTEIN XRCC3

**Phillip Connell, Nazli Siddiqui,
Sara Hoffman, Audrey Kuang,
Ralph R. Weichselbaum, and Douglas K. Bishop**

Department of Radiation and Cellular Oncology,
University of Chicago

dbishop@midway.uchicago.edu

Genetic studies have shown that cells require Xrcc3 protein (Xrcc3p) for DNA repair via the homologous recombination repair (HRR) pathway. Cells lacking Xrcc3p are, consequently, 50-100 fold more sensitive to cross-linking chemotherapeutic drugs. Based on these data, phage display was used to develop dodecapeptides that directly bind Xrcc3 protein and inhibit HRR. Two consecutive rounds of phage display were performed and identified several Xrcc3p-binding peptide sequences. Several of these peptides displayed sequence similarity to Rad51C protein, a known binding partner of Xrcc3p. This portion of Rad51Cp (amino acids 14-25) was synthesized in fusion with a membrane transduction domain. The resulting peptide, Rad51C(14-25)-PTD4, was delivered to chinese hamster ovary (CHO) cells and shown to be capable of inhibiting sub-nuclear assembly of the central HRR protein, Rad51, following DNA damage. Colony forming assays showed that the peptide also sensitized cells to the lethal effects cisplatin. Identical experiments performed with a scrambled version of this peptide as a negative control showed no effect on Rad51 focus formation or sensitivity to cisplatin. Furthermore, CHO cells defective in Xrcc3p expression were unaffected by the Rad51C(14-25)-PTD peptide. The activity of the peptide was also tested on three human breast tumor lines MCF7, BT20, and MDA MB-231. To date, no activity has been detected in these lines and efforts are underway to isolate improved inhibitory peptides.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0196 supported this work.

INTERACTIONS BETWEEN BRCA2 AND RAD51 GOVERN HOMOLOGOUS RECOMBINATIONAL REPAIR IN HUMAN CELLS

Mark A. Brenneman,[1] Xu Guo,[1] Brant M. Wagener,[1] Cheryl A. Miller,[1] Zhiyuan Shen,[1] David J. Chen,[2] and Jac A. Nickoloff[1]

[1]Department of Molecular Genetics and Microbiology, University of New Mexico School of Medicine; and [2]Life Sciences Division, Lawrence Berkeley National Laboratory

mbrenneman@salud.unm.edu

The breast cancer susceptibility gene BRCA2 has a critical involvement in cellular response to DNA damage, and is required for maintenance of genomic stability. Demonstration of direct interactions between BRCA2 protein and the mammalian RAD51 strand transfer protein suggested that the BRCA2 protein might participate in homologous recombinational repair (HRR) of DNA double-strand breaks, and possibly other forms of damage associated with arrested DNA replication.

Two regions of BRCA2 mediate interaction with RAD51; an extreme C-terminal domain encoded by exon 27, and the eight "BRC repeats" encoded by exon 11. In transgenic mice, deletion of all the RAD51-interacting domains of BRCA2 causes embryonic lethality. A less severe phenotype is seen with BRCA2 truncations that preserve some, but not all, of the BRC repeats. These mice can survive beyond weaning, but are runted, infertile, and die very young from cancer. Cells from such mice show hypersensitivity to some DNA-damaging agents and chromosomal instability. Mice with a deletion of only exon 27 have a shorter life span than control littermates, but no other apparent phenotype, indicating that exon 27 deletion is less severe than truncations that delete some BRC repeats. At the cellular level however, deletion of exon 27 causes reduced viability, hyper-sensitivity to the DNA interstrand cross-linking agent mitomycin C, and high frequencies of chromosomal breakage and rearrangements, much like more severe truncations. Thus, the extreme carboxy-terminal region encoded by exon 27 is important for BRCA2 function, probably because it is required for a fully functional interaction between BRCA2 and RAD51. In human cells, expression of peptides containing a single BRC repeat cause hypersensitivity to ionizing radiation (IR) or cisplatin, a reduction in the formation of subnuclear RAD51 foci after IR exposure, and suppression of the G2/M cell cycle checkpoint. As with deletion of exon 27 in mouse cells, this 'dominant-negative' effect of single BRC repeats in human cells is presumed to come about by disruption of normal protein-protein interactions between BRCA2 and RAD51.

We tested the dependence of HRR upon RAD51/BRCA2 interaction, in human cells containing a reporter substrate that permits direct measurements of HRR of a specifically induced chromosomal double-strand break (DSB). Frequencies of DSB-induced HRR were measured in cells with and without inhibition of RAD51/BRCA2 interaction by transient transfection with vectors encoding RAD51-interaction domains of BRCA2. Expression of a peptide containing BRC repeat #1 during DSB induction caused the frequency of HRR to drop by more than 20-fold. Expression of the carboxy-terminal domain encoded by exon 27 resulted in smaller but significant reductions in HRR frequency. The results confirm that BRCA2 directly influences HRR, and does so through interactions with RAD51 that involve both the BRC repeats within exon 11, and the extreme C-terminus of BRCA2 encoded by exon 27. The impairment of this critical DNA repair pathway, and resulting genomic instability, in cells that lack full-length BRCA2 may largely explain the tumorigenic effects of BRCA2 mutation. The results further suggest that, in addition to BRCA2, RAD51 and other HRR-associated proteins may be useful diagnostic and prognostic markers in sporadic as well as hereditary cancer, and potential targets for individualized cancer therapy.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0367 supported this work.

SMALL CHEMICAL MOLECULES THAT DISRUPT BRCA2 AND RAD51 INTERACTION FOR ADJUVANT BREAST CANCER THERAPY

Chen P-L

Department of Molecular Medicine, University of Texas
Health Science Center at San Antonio, San Antonio, TX 78245

chenp0@uthscsa.edu

Mutations in the BRCA2 gene have been found in many familial breast cancers. Cells deficient in BRCA2 or containing BRCA2 mutations lead to various cellular abnormalities including increased sensitivity to DNA damage by genotoxic agents, accumulation of DNA double-strand breaks, chromosome abnormalities, and changes in cell cycle check point and apoptotic responses. It was proposed that BRCA2 could be involved in DNA repair response. This hypothesis was further supported by my observation that BRCA2 interacts with Rad51, a protein involved in DNA recombination repair through six highly conserved BRC repeats. In previous work, we demonstrated that the interaction between BRCA2 and Rad51 mediated by BRC repeats is critical for cellular response to DNA damage. In facts, breast cancer with BRCA1 or 2 mutation exhibits hypersensitivity to radiation therapy. However, more than 90% of breast cancers has no mutation in either BRCA1 or 2. To exploit the importance of this interaction toward the development of new anti-breast cancer agents, we plan to isolate small molecules that disrupt the interaction between BRCA2 and Rad51. We will isolate small molecules which disrupt the interactions between BRCA2 and Rad51 using reverse yeast two-hybrid screening and then to evaluate the ability of the identified molecules to sensitize cultured breast cancer cells to the genotoxic and cytotoxic effects of ionizing radiation. Finally, we will assess the efficacy of the small molecules as adjuvant chemotherapy in the radiation treatment of mouse mammary carcinoma. We have isolated 8 lead chemical molecules which interupt the interaction between BRC repeat and Rad51 in a yeast two-hybrid system.

The above experiments represent the first attempts to evaluate the potentials of small molecules that disrupt interaction between BRCA2 and Rad51 for serving as an anti-breast cancer adjuvant. The new drugs that interfere with the repair machinery in cancer cells may render them hypersensitive to radiation. Determining the in vivo effects of small molecules identified in this study could be an important step in combating resistance to radiation therapy.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0409 supported this work.

ROLES OF BRCA2 IN HOMOLOGOUS RECOMBINATIONAL REPAIR

Yi-Ching Lio, Mark Brenneman, Jac A. Nickoloff, and David J. Chen

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

ylio@lbl.gov

The BRCA2 tumor suppressor protein is a major contributor to a dominantly inherited predisposition to breast cancer. Recent evidence has implicated a role of BRCA2 in genomic stability and in homologous recombinational repair (HRR) of DNA double-strand breaks. It has been shown that BRCA2 physically interacts with Rad51, a key protein in HRR. Our works aim to provide direct experimental evidence for the functions of BRCA2 in the HRR pathway.

Using an artificial reporter system in human cells, we have measured the capability for HRR with and without disruption of the BRCA2-Rad51 interaction through expression of various fragments of BRCA2. Expression of a BRC repeat of BRCA2 was found to suppress the HRR frequency for chromosome breaks. To understand how BRCA2 regulates HRR, we have expressed and purified three fragments of BRCA2. We found that a small fragment of BRCA2 displays an inhibitory effect on the biochemical activity of Rad51. The effects of large fragments of BRCA2 on Rad51 will be further determined. Five human Rad51 paralogs have been recently identified and suggested a mediating role in HRR. We are using a novel biochemical strategy to examine whether BRCA2 interacts simultaneously with Rad51 and Rad51 paralogs to form a multiprotein complex and whether BRCA2 regulates the complex formation of Rad51 paralogs.

The work will direct toward elucidating the mechanism of actions of BRCA2 in DNA repair and the mechanism underlying the pathogenesis of BRCA2-mediated breast cancer. Such an understanding would reveal new targets for therapeutic intervention in breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0542 and DAMD17-99-1-9170 supported this work.

BRCA2 AND THE DNA DOUBLE-STRAND BREAK-REPAIR MACHINERY

Chen P-L, Xiao J, Liu C, and Lee W-H

Department of Molecular Medicine,
University of Texas Health Science Center at San Antonio,
San Antonio, TX 78245

chenp0@uthscsa.edu

Mutations in the BRCA2 gene have been found in many familial breast cancers. Cells deficient in BRCA2 or containing BRCA2 mutations lead to various cellular abnormalities including increased sensitivity to DNA damage by genotoxic agents, accumulation of DNA double-strand breaks, chromosome abnormalities, and changes in cell cycle check point and apoptotic responses. We proposed to test the model, BRCA2 modulates the early steps of repair mediated by the Rad50 nuclease complex, and the later stages catalyzed by the Rad51 recombinase. To test this hypothesis, we proposed to establish the specificity of the interactions between Rad50, RINT-1 and BRCA2 and then to determine how BRCA2 influences double strand break repair machinery.

A novel 87 kDa protein named RINT-1 was identified using the C-terminal region of hRad50 as the bait in a yeast two-hybrid screen. Human RINT-1 shares sequence homology with a novel protein identified in Drosophila melanogaster, including a coiled-coil domain within its N-terminal 150 amino acids, a conserved central domain of about 350 amino acids, and an C-terminal region of 90 amino acids exhibiting 35-38% identity. The conserved central and C-terminal regions of RINT-1 are required for its interaction with Rad50 and BRCA2. While BRCA2 utilize its conserved region to bind RINT-1. RINT-1 expressed throughout the cell cycle and specifically binds to Rad50 only during late S and G2/M phases, suggesting that RINT-1 may be involved in cell cycle regulation. Further, MCF-7 cells expressing the N-terminally truncated RINT-1 displayed a defective radiation-induced G2/M checkpoint, which implies that RINT-1 may involve in the regulation of cell-cycle control after DNA damage.

The above experiments represent the first attempts to test a new idea concerning the part that BRCA2 plays in DNA double-strand break repair. Demonstrating a dual role for BRCA2 in the DNA-repair process represents a paradigm shift concerning its involvement in tumorigenesis and how this knowledge can be exploited toward the development of new strategies for its prevention and treatment of breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9398 supported this work.

INVOLVEMENT OF 53BP1, A P53-BINDING PROTEIN, IN THE DNA DAMAGE RESPONSE

Bin Wang[1] and Stephen J. Elledge[1,2,3]

[1]Verna and Marrs McLean Department of Biochemistry and Molecular Biology; [2]Howard Hughes Medical Institute; and [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030

selledge@bcm.tmc.edu

53BP1 binds to the tumor suppressor p53 and may be involved in activating transcription of its downstream target genes. In response to γ-irradiation (IR), 53BP1 immediately relocalizes and forms discrete nuclear foci. 53BP1 is also phosphorylated in response to IR in an ataxia-telangiectasia-muated (ATM)-dependent manner. Since 53BP1 is involved in the early cellular response to IR, it is likely to play an important role in DNA damage response.

We are investigating the function of 53BP1 in DNA damage response using both siRNA approach to inhibit 53BP1 in cells and generating conditional deletion of 53BP1 gene in mice. We have found that 53BP1 is essential for cell proliferation that inhibition of 53BP1 by siRNA will lead to cell death. 53BP1-inhibited cells are slow in S-phase progression after cells are synchronized and released from the double-thymidine block. To further study the role of 53BP1 in embryonic development and tumor suppression, we also made a 53BP1 conditional knockout vector that will replace the endogenous allele of 53BP1 with a conditional allele that has two loxP sites encompassing the second exon of the 53BP1 gene. Thus the second exon will be excised upon introduction of the recombinase, cre. This will result in a frame shift and generate a small peptide that is likely to behave as a null allele. We are generating the murine ES cells that contain a conditional 53BP1-deficient allele and will use that to derive a mouse containing a conditional 53BP1 allele, and study the physiological role of 53BP1 in embyrogenesis and tumor suppression. So far, our study has shown that 53BP1 is a important component in DNA response pathway in mammalian cells. It will shed a light on the understanding of the fundamental alterations in breast cancer development.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0134 supported this work.

HUMAN RAD51 INVOLVEMENT IN GENOMIC INSTABILITY AND DEVELOPMENT OF BREAST CANCER

Schmutte C., Tombline G., Slupianek A., Skorski T., and Fishel R.

Thomas Jefferson University, Kimmel Cancer Center

cschmutte@lac.jci.tju.edu; rfishel@lac.jci.tju.edu

Genomic instability is a common denominator of human cancers. Several genetic defects have been identified that result in genomic instability and hereditary and sporadic tumors. Approximately 10% of breast cancers display a hereditary predisposition. Germline alterations in human BRCA1 and BRCA2 genes account for this pattern roughly equally. Extensive screening of human breast tumors have shown that BRCA1/2 mutations in sporadic breast cancer are very rare. A possible explanation for this inconsistency is that other gene products which interact with the BRCA2 pathway may be inactivated during breast cancer development. Several recent findings have drawn attention to hRAD51 as such a candidate. hRAD51 is a member of a family of proteins which are instrumental in DNA repair by homologous recombination (HR). In this study we first examined the hRAD51 gene in tumors with 15q14-15 deletions, the location of hRAD51. We did not find any changes compared to normal tissue which supports the notion that hRAD51 is an essential gene.

While bacteria seem to require only one member of the RecA/RAD51 protein family, the human genome encodes seven of these proteins which may function alone or in a complex with each other. To further characterize the HR pathway in human cells we tested for possible interactions between hRAD51 and the five hRAD51 paralogs involved in HR as well as interactions between these proteins and BRCA2, the BRCA1-interacting protein BARD1 and other proteins suspected to function in HR. We detected a network of strong interactions suggesting stable complexes, and weaker interactions. Some of these weaker interaction signals between hRAD51 paralogs were modified by ATP and ADP which may indicate a regulatory role for adenosine nucleotides.

We have also examined the role of hRAD51-dependent DNA repair by homologous recombination in BCR/ABL-expressing cells. We found that the oncogenic tyrosine kinase BCR/ABL upregulates hRAD51 and several hRAD51 paralogs. Elevated DNA repair by recombination seems to be a major pathway by which BCR/ABL-expressing cells become drug resistant. These findings may have significant implications for cancer therapy.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9406 supported this work.

OXIDATIVE DAMAGE, CYP1B1, AND BREAST CANCER

M. L. Russell, C. A. Erdmann, D. Li, and R. Goth-Goldstein

Lawrence Berkeley National Laboratory,
M.D. Anderson Cancer Center

r_goth-goldstein@lbl.gov

The study addresses the initiating causes and early events of breast cancer. Increased oxidative DNA damage has been reported in breast tissue from breast cancer patients. This study investigates the role of the cytochrome P450 enzyme CYP1B1 in formation of oxidative damage. The CYP1B1 enzyme is involved in the metabolic activation of environmental carcinogens, and also metabolizes estrogen to a carcinogenic metabolite. We have found previously that CYP1B1 is expressed in breast and that expression was significantly higher in non-tumor breast tissue from breast cancer patients than from healthy individuals, suggesting a possible role of CYP1B1 in the etiology of breast cancer. The concept tested is that high levels of CYP1B1 lead to increased oxidative damage, thereby initiating the carcinogenic process. CYP1B1 expression and oxidative damage are being determined in a collection of breast tissue specimens from reduction mammoplasties and non-tumor peripheral tissue from mastectomies and the relationship of CYP1B1 expression and oxidative DNA damage is investigated. CYP1B1 expression is determined by a semiquantitative RT-PCR relative to the beta-actin gene. 8-hydroxy-2'deoxyguanosine (8-oxoG), is used as a reliable index of overall oxidative DNA damage and is determined by 32P-postlabeling. The relationship between CYP1B1 expression and 8-oxoG levels will be examined and the influence of CYP1B1 and oxidative damage on risk of breast cancer will be evaluated.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0543 supported this work.

EOSINOPHIL PEROXIDASE AND OXIDATIVE DAMAGE OF DNA IN BREAST CANCER

Stanley L. Hazen, M.D., Ph.D., and Zhongzhou Shen, Ph.D.

Cleveland Clinic Foundation

hazens@ccf.org

A considerable body of evidence implicates reactive oxygen species in promoting DNA oxidative damage and the development of breast cancer. For example, levels of mutagenic hydroxyl radical (OH) - dependent base modifications are significantly increased in DNA isolated from invasive ductal carcinoma of the female breast. The source of oxidizing equivalents (e.g., H2O2 and O2·-) and the pathways for production of OH and other oxidizing species in breast cancer are not established. We hypothesize that one potential source of endogenous reactive oxygen species that has not yet been considered is eosinophils. Eosinophils are phagocytic cells that are optimized for the production of reactive-oxygen, -halogen and free radical species including OH. Recent immunohistological studies demonstrate that 88% of human breast carcinomas contain eosinophil peroxidase (EPO) deposits in and around the tumor.

Since EPO is the only known human enzyme that selectively generates brominating species, we hypothesized that brominated bases may serve as "molecular fingerprints" for identifying sites of EPO-catalyzed DNA oxidative damage. Using an array of chemical approaches, we have shown that EPO effectively uses plasma levels of bromide as co-substrate to brominate bases in nucleotides and double stranded DNA forming several stable novel brominated adducts. Products were characterized by HPLC with on-line UV spectroscopy and electrospray ionization tandem mass spectrometry (LC/ESI/MS/MS). We have identified a novel free transition metal ion-independent mechanism for damage of cellular DNA, RNA and cytosolic nucleotides by activated neutrophils and eosinophils. The mechanism involves reaction of peroxidase-generated hypohalous acid (HOCl or HOBr) with intracellular superoxide (O2·-) forming OH, a reactive oxidant species implicated in carcinogenesis. Collectively, our results identify leukocyte peroxidases as potential contributors to the well-established link between inflammation, DNA damage and cancer development. The present results also suggest that specific brominated DNA bases may serve as novel and specific markers for monitoring oxidative damage of DNA and the nucleotide pool by EPO-generated brominating oxidants.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9133 supported this work.

BIOCHEMICAL ACTIVITIES OF THE BREAST CANCER PREDISPOSITION PROTEIN BRCA2

Wolf-Dietrich Heyer, Tammy Doty, and Olga Miroshnychenko[1]

Section of Microbiology and Center for Genetics and Development, University of California, Davis, Davis, CA 95616; [1]Present address: Department of Cell and Molecular Biology, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

wdheyer@ucdavis.edu

Mutations in the human BRCA2 gene predispose to breast, ovarian and other cancers. The 384 kDa 3,418 amino acid Brca2 protein has been implicated in the maintenance of genomic stability through a function in recombinational DNA repair. Presumably this involves a direct interaction between Brca2 protein and the Rad51 homologous pairing and DNA strand exchange protein. The purpose of this project is to purify and analyze human Brca2 protein or significant parts of it to understand its interaction with DNA and its functional interplay with human Rad51 protein during recombinational DNA repair. The project aims at defining the biochemical properties of Brca2 protein with regards to its presumed function in DNA metabolism alone and in conjunction with the known recombinational repair protein Rad51.

We have overexpressed in the yeast Saccharomyces cerevisiae and purified to apparent homogeneity a 568 amino acid fragment from the C-terminal region of human Brca2 protein fused to glutathion-S-transferase (GST). Preliminary biochemical analysis of the fusion protein failed to detect any DNA binding activity. We have also succeeded in overexpressing the apparent full-length Brca2 protein fused to GST. A significant portion of the apparent full-length fusion protein is soluble and produced in sufficient quantity for preparative purification. We are presently pursuing the purification of this fusion protein. Using DNA binding and an in vitro recombination assay (DNA strand exchange reaction with circular single-stranded and linear double-stranded DNA) catalyzed by human Rad51 protein, we intend to characterize the biochemical properties of the human Brca2 protein.

The biochemical analysis of purified Brca2 tumor suppressor protein is critical to elucidate its molecular function and to design quantitative in vitro assays for Brca2.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0187 supported this work.

ANTICANCER AGENT ADOZELESIN INDUCES RPA FOCUS FORMATION AND PHOSPHORYLATION IN S PHASE CELLS

Shu-Ru Kuo, Jen-Sing Liu, Terry A. Beerman, and Thomas Melendy

Department of Microbiology, University at Buffalo, State University of New York School of Medicine and Biomedical Science, Buffalo, NY 14214

shurukuo@acsu.buffalo.edu

Adozelesin belongs to a family of alkylating minor groove DNA binders. It alkylates the N3 of adenine without causing DNA strand breaks. In cells treated with adozelesin, DNA replication is inhibited. Using cell-free SV40 DNA replication assays, we have demonstrated that replication protein A (RPA) is inactivated in cells treated with adozelesin. RPA is the major eukaryotic single-stranded DNA binding protein that is essential for DNA replication and plays important role in many types of DNA repair. Interestingly, while RPA's function in DNA replication is inactivated in adozelesin-treated cells, it remains active for repair synthesis. This selective regulation of RPA suggests it to be a unique effector in S phase checkpoint pathways. RPA is also phosphorylated and forms focal points in response to DNA damage. We have therefore used RPA phosphorylation and focalization as markers to further study the cellular responses to adozelesin-induced DNA damage.

In asynchronously cultured cells, adozelesin induces DNA damage markers only in a portion of cells. Detailed studies revealed that those cells that showed DNA damage-induced change in RPA are in the S phase of cell cycle. More specifically, DNA replication progression is required for adozelesin-induced RPA phosphorylation and focalization. In cells deficient in either ATM or its related protein ATR, the intensity of adozelesin-induced RPA focalization, which can be seen within minutes of treatment, is comparable to cells with functional ATM and ATR. However, in ATM or ATR deficient cells, adozelesin-induced RPA phosphorylation cannot be detected until much later times and the intensity was dramatically reduced. These results suggest that multiple cellular checkpoint mechanisms are induced in cells treated with adozelesin.

Our results suggest that adozelesin selectively triggers DNA damage checkpoints in S phase cells. Considering the high growth rate of tumor cells, chemotherapeutic agents that target on either S or M phase of cell cycle certainly will have higher impact on malignant tissues. To further understand the action of cell cycle specific anti-cancer drugs will provide a good guideline for better therapeutic agents design with higher efficacy and lower side effect.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0418 supported this work.

MOLECULAR ETIOLOGY OF DNA REPAIR DEFICIENCY IN NOVEL NON-TUMOR-ADJACENT AND TUMOR CELL LINES

Jean J. Latimer, Crystal M. Kelly, Janiene A. Patterson, Sharon Wenger, Victor Vogel, Stephen G. Grant, and Jennifer Johnson

Department of Obstetrics, Gynecology, and Reproductive Science, University of Pittsburgh

latimerj@pitt.edu

We have developed a novel in vitro primary Human Mammary Epithelial Culture (HMEC) system for normal breast tissue and tumors (www.pitt.edu/~rsup/mgb/latimer.html). We have generated 90 cell lines derived from normal human breast epithelium, breast tumors stages 0-IV,and matching histologically "normal" non-tumor adjacent (NTA) tissue. Using a functional assay, we have shown that loss of Nucleotide Excision Repair (NER) is intrinsic to breast tumorigenesis. Further, we have shown that 85% of the matching NTA cultures manifest the same NER deficiency as the tumor. We hypothesize that the loss of NER capacity in these cultures is due to inactivation of one or more of the genes in the NER pathway. We have therefore analyzed the expression of the known NER genes using RNAse protection. Preliminary data have implicated several genes in the deficiency in DNA repair manifested in these cultures, including genes that all code for proteins with DNA helicase activity or are part of the unwinding complex. Since all of the heritable diseases associated with NER deficiency are genetically recessive, the mechanism of inactivation of these genes during carcinogenesis should involve allelic loss of heterozygosity (LOH). We are currently karyotyping these cultures, specifically looking for alterations consistent with LOH at the known NER loci. Of particular interest should be the results in NTA cultures, which should not have accunulated the karyotypic abnormalities characteristic of tumor progression. Using these techniques, we should be able to detect allele loss at any of the known NER genes. We have begun the karyotyping on two of our non-diseased breast reduction cell lines, JL BRL 11 and JL BRL 14 at passages 7 or beyond and both are overtly normal.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0681 supported this work.

MITOTIC PHOSPHORYLATION OF THE BREAST CANCER SUSCEPTIBILITY GENE 2, BRCA2

Horng-Ru Lin and Wen-Hwa Lee

Department of Molecular Medicine/Institute of Biotechnology, The University of Texas Health Science Center at San Antonio, TX

leew@uthscsa.edu

Approximately one in ten women in Western countries will develop breast cancer. 5-10 % of all breast cancer cases is inherited. Mutations in human breast cancer susceptibility genes, BRCA1 and BRCA2, are responsible for a largely proportion of hereditary early onset breast cancer. BRCA1 plays key roles in DNA damage response and repair through interaction with Rad50/Mre11/NBS1 complex. It also plays a regulatory role in transcriptional regulation. BRCA2 may participate in maintenance of genomic integrity through its interaction with RAD51, which is required for DNA repair and genetic recombination. A possible role for BRCA2 in modulating specific events in M phase that are required to ensure the fidelity of mitotic chromosome segregation has been proposed. Here, we found that BRCA2 is hyperphosphorylated specifically during M phase in the presence or absence of nocodazole treatment, a microtubule-disrupting agent that arrests cells in prometaphase or metaphase by activating mitotic spindle checkpoint, and becomes dephosphorylated after removal of nocodazole and leaving the M phase. In addition, the hyperphosphorylation of BRCA2 observed during mitosis is not detected upon DNA damage. These preliminary observations implicate that BRCA2 may have a role in modulating early events of mitosis that is regulated by posttranslational modification. Further studies are warranted to elucidate the precise role of BRCA2 in chromosome segregation during M phase.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9402 supported this work.

DYNAMIC ASSOCIATION OF BRCA1 WITH HOMOLOGOUS RECOMBINATION PROTEINS IN PML NUCLEAR BODIES DEPENDS ON NBS1 IN HUMAN ALT CELLS

Guikai Wu,[1] Xianzhi Jiang, Wen-Hwa Lee, and Phang-Lang Chen

Department of Molecular Medicine and Institute of Biotechnology, University of Texas Health Science Center at San Antonio, TX 78245

wug@uthscsa.edu

Mammalian immortalized cells maintain their telomere lengths through a telomerase-dependent or an independent pathway that is also known as alternative lengthening of telomeres pathway (ALT). In human ALT cells, proteins playing crucial roles in DNA replication and homologous recombination along with telomere DNA and telomere binding proteins were found in PML nuclear body (also known as ALT-associated PML bodies, APBs), implicating a recombination-mediated mechanism for telomere lengthening. Here we showed that BRCA1, similar to other recombination proteins, localizes to APBs in human ALT cells specifically during late S/G2 phase. Co-localization of BRCA1 with Rad50/Mre11 complex in APBs requires the intact Nijmegen breakage syndrome protein, NBS1, because the N-terminally BRCT domain-deleted NBS1 failed to recruit this complex to APBs. However, this process is independent of telomere binding factor TRF1, suggesting a central role of NBS1 in recruiting Mre11 and BRCA1 to APBs. Localization of the recombination repair protein, Rad51, in APB is independent of those nuclear dots induced by ionizing irradiation. Although Rad50/ Mre11/NBS1 complex are involved in both telomerase-dependent and ALT pathway, BRCA1 appears to be only required for ALT pathway. These results suggest that BRCA1 is a component of APBs and has a novel role in mammalian ALT pathway.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9402 supported this work.

REPLICATION AND DNA DAMAGE-INDUCED PHOSPHORYLATION OF HUMAN RAD17 BY ATR IS REQUIRED FOR CHECKPOINT ACTIVATION

Sean Post, Yi-chinn Weng, and Eva Y.-H. P. Lee

Department of Molecular Medicine/Institute of Biotechnology,
University of Texas Health Science Center at San Antonio

post@uthscsa.edu

Breast cancer affects 1 in 9 women in the United States. Most breast cancers arise from sporadic, not familial, genetic origins; therefore, understanding the mechanism of breast cancer development is paramount to prevention. One of the quintessential characteristics of cancerous cells is the loss of cell cycle checkpoint control.

The focus of this work addresses the roles of the checkpoint Rad proteins in checkpoint activation in response to DNA damage and replication. Our data, obtained from breast and ovarian cancer cell lines, as well as mice, demonstrates a functional link between aberrant DNA structures, signal transduction, and checkpoint activation.

ATR (ataxia telangiectasia-mutated, ATM, and Rad3-related) is a protein kinase required for both DNA damage-induced cell cycle checkpoint responses and the DNA replication checkpoint that prevents mitosis prior to the completion of DNA synthesis. Although, ATM and ATR kinases share many substrates, the different phenotypes of ATM- and ATR-deficient mice demonstrate that these kinases are not functionally redundant. Here we demonstrate that ATR but not ATM phosphorylates the human Rad17 (hRad17) checkpoint protein on Ser^{635} and Ser^{645} in vitro. In undamaged synchronized human cells, these two sites are phosphorylated in late G1, S, and G2/M, but not in early-mid G1. Treatment of cells with genotoxic stress induced phosphorylation of hRad17 in cells in early-mid G1. Additionally, Rad17 is phosphorylated in actively replicating but not quiescent wild type and ATM-deficient murine tissues. These results demonstrate that the kinase activity of ATR on hRad17 is activated during normal replication and by perturbation to the DNA.

Expression of kinase-inactive ATR results in reduced phosphorylation of these residues, but these same serine residues are phosphorylated in IR-treated ATM-deficient human cell lines. Similar results were found in vivo in ATM-deficient mice. Furthermore, expression of a hRad17 mutant, with both serine residues changed to alanine, abolished IR-induced activation of the G1/S checkpoint in the breast cancer cell line, MCF-7. These results suggest ATR and hRad17 are essential components of an ATM-independent DNA damage response pathway in mammalian cells.

These data are the first to indicate that the checkpoint Rad proteins may play a role in normal DNA synthesis. We believe that understanding the consequences of the loss of the cell cycle checkpoints may lead to a better comprehension of cancer development and useful therapies in the future.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9402 supported this work.

A TARGETED INHIBITION OF A KEY DNA REPAIR ENZYME IN BREAST CANCER TREATMENT

Chung H. Kim, Sue Park, and Suk-Hee Lee

Department of Biochemistry and Molecular Biology,
Walther Oncology Center and Indiana University Cancer Center, Indiana University School of Medicine,
Indianapolis, IN 46202

slee@iupui.edu

Our ultimate goal is to develop a novel approach to breast cancer treatment by targeting proteins involved in damage-signaling pathway and/or DNA repair, which would greatly facilitate tumor cell cytotoxic activity and programmed cell death upon DNA damaging drug treatment. By inhibiting DNA repair pathways that tumor cells rely on to escape chemotherapy, we expect not only to facilitate cell killing, but also to reduce the severity of the associated toxicity. DNA dependent protein kinase (DNA-PK) is a nuclear serine/threonine protein kinase, not only essential for DNA repair but also involved in sensing and transmitting damage signal to downstream targets, which eventually contribute to the cell cycles arrest. We hypothesize that targeted inhibition of DNA-PK sensitizes breast cancer cells. To test this, we have developed a specific peptide that mimics the domain DNA repair protein essential for protein-protein interaction. Preliminary study indicates that this peptide selectively target and disrupt specific repair pathway. More importantly, a peptide-based inhibitor with target sequence can effectively sensitize breast cancer cells only in the presence of DNA damage, suggesting that the sensitization of cancer cells occurs through blockade of DNA repair pathway. Further study would be necessary to validate the feasibility of the anticancer targets and to develop vital lead compounds for future pharmaceutical development.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0295 supported this work.

BREAST CANCER SUSCEPTIBILITY GENES AND GENOMIC INSTABILITY

Wen-Hwa Lee, Ph.D.

Department of Molecular Medicine/Institute of Biotechnology, The University of Texas Health Science Center at San Antonio, TX

leew@uthscsa.edu

BRCA1 and BRCA2 are two known breast cancer susceptibility genes. Mutations within BRCA1 and BRCA2 are responsible for most familial breast cancer cases. Targeted deletion of Brca1 or Brca2 in mice has revealed an essential function for their activity in cell proliferation during embryogenesis. Human cancer cells and mouse cells deficient in BRCA1 or BRCA2 exhibit radiation hypersensitivity and chromosomal abnormalities, thus revealing a potential role for both BRCA1 and BRCA2 in the maintenance of genetic stability through participation in the cellular response to DNA damage. Functional analyses of the BRCA1 and BRCA2 gene products have established their dual participation in transcription regulation and DNA damage repair. These results provide a biochemical basis for their inherent tumor suppressor properties.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9402 supported this work.

UBIQUITINATION OF RNA POLYMERASE II AND ITS ROLE IN TRANSCRIPTION AND DNA REPAIR

Keng-Boon Lee,[1] Dong Wang,[2] Stephen J. Lippard,[2] and Phillip A. Sharp[1,3]

[1]Center for Cancer Research and Department of Biology, [2]Department of Chemistry, and [3]McGovern Institute for Brain Research, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139

kbl@mit.edu

The large subunit of RNA polymerase (Pol) II is ubiquitinated in cells upon DNA damage from UV radiation and *cis*platin treatment. These DNA lesions block transcription and are removed by transcription-coupled repair (TCR), *i.e.,* the preferential repair of lesions in the transcribed DNA strand. Cockayne Syndrome cells impaired in TCR are deficient in UV-induced ubiquitination of polymerase, supporting a role for ubiquitination in DNA repair and transcription. A relevance to the cancer treatment is also implicated since *cis*platin is a chemotherapeutic agent effective against solid tumors like testicular and breast tumors. Ubiquitination of Pol II could be related to the removal of the stalled polymerase during DNA repair, facilitating the access of repair enzymes to the site of lesion.

There is increasing evidence which suggest that TCR may play a critical role in development, and cancer and its treatment. To understand the molecular processes that occur when a transcribing Pol II is obstructed by a DNA lesion, we examined the role of Pol II ubiquitination in transcription and DNA repair of cisplatin/DNA adducts, using an *in vitro* ubiquitination assay with HeLa nuclear extracts. In our recent publication at the Proceedings of the National Academy of Sciences, we report the first evidence of a correlation between ubiquitination of RNA Pol II and the arrest of transcription *in vitro*. We show that ubiquitination of Pol II is significantly induced by alpha-amanitin, an amatoxin which blocks Pol II elongation and causes its degradation in cells. Pol II undergoes similar ubiquitination on DNA containing cisplatin adducts that arrest transcription. Stimulation of ubiquitination requires the addition of template DNA, and does not occur in the presence of an antibody to the general transcription factor TFIIB, indicating the transcription dependence of the reaction. We propose that components of the reaction recognize elongating polymerase-DNA complexes arrested by alpha-amanitin or cisplatin lesions, triggering ubiquitination.

Our results confirm that Pol II ubiquitination is induced by the arrest of transcription, suggesting a role in signaling for TCR. The absence of TCR leads to an increased incidence of mutation, and transcription inhibition, due to an RNA Pol II stalled at the site of lesion. TCR is also likely to be important in the efficacy of cancer chemotherapeutic agents which cause DNA lesions. Breast cancer susceptibility gene BRCA1-deficient cells are known to be defective in transcription-coupled repair of oxidative DNA damage, and these cells are prone to malignant transformation in a p53-deficient background, resulting in breast cancer. Elucidating the cellular events that occur in response to DNA damage of transcribed genes (TCR), will provide an insight into some of the molecular interactions that cooperate to transform cancer-predisposed breast cells into malignancy and improve an understanding of the disease, facilitating better prevention and treatment the future.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0314 supported this work.

GENETIC AND EPIGENETIC ALTERATIONS OF MISMATCH REPAIR GENES IN SPORADIC BREAST CANCER WITH MICROSATELLITE INSTABILITY

Guo-Min Li, Hiroaki Murata, Nada H. Khattar, Yuna Kang, and Liya Gu

Department of Pathology, University of Kentucky

gmli@uky.edu

Breast cancer is the most prevalent malignancy in women who live in western industrialized nations. The cause of this disease is still unknown even though two breast cancer susceptibility genes (BRCA1 and BRCA2) have been identified, which are mutated in approximately 5-10% of all breast cancers. Recent studies indicate that a substantial fraction of breast tumors have frequent microsatellite instability (MSI), a phenomenon tightly associated with mismatch repair (MMR) deficiency that was initially observed in cells from hereditary non-polyposis colorectal cancers (HNPCC) and some sporadic colorectal cancers. The identification of MSI in breast cancer suggests that breast cancer may be associated with mismatch repair defects. To test this hypothesis, we investigated MSI, expression of two critical MMR genes hMSH2 and hMLH1, and genetic and epigenetic modifications of these two genes in 32 sporadic breast tumors. MSI was identified in 15 cases. Immunohistochemistry analysis revealed that all cases but one with MSI had lower than normal expression of hMSH2 (9 cases), hMLH1 (12 cases), or both (7 cases). Both genetic and epigenetic modifications of these MMR genes have been identified in MSI breast tumors. Seven of these MSI cases harbored mutations or polymorphisms in hMSH2 and hMLH1, and 10 of them exhibited hypermethylation in the promoter region of hMLH1. These results suggest that both genetic and epigenetic alterations of hMSH2 and/or hMLH1 contribute to genomic instability and tumorigenesis in sporadic breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9246 supported this work.

ANALYSIS OF RAD9'S BRCT DOMAINS: ROLES IN THE CHECKPOINT RESPONSE AND DNA DAMAGE REPAIR

Kara Nyberg and Ted Weinert

University of Arizona, Molecular and
Cellular Biology Department, Tucson, AZ 85721-0106

knyberg@email.arizona.edu

Brca1 is a checkpoint protein commonly abrogated in families affected with breast and ovarian cancers. Insight into BRCA1 function can be gained by studying the putative budding yeast homologue, RAD9. Rad9 and Brca1 perform some similar functions, and RAD9 shares partial homology with BRCA1, as both possess BRCA1 Carboxyl Terminus (BRCT) domains commonly found in cell cycle checkpoint and DNA repair proteins.

To study the BRCT domains in Rad9, deletion constructs were created. These strains were assayed for their ability to recognize and repair two forms of DNA damage, UV exposure and methyl methane sulfonate (MMS), and for their ability to arrest in response to DNA damage. Other indicators, such as phosphorylation of Rad53, were also analyzed as signs of activation of the rad9 deletion constructs.

Our results reveal that the BRCT domains are solely required for Rad9 homodimer formation during the G2/M-phase of the cell cycle. The homodimer state is required for UV repair and an arrest response in the presence of DNA damage when Rad9 is expressed at endogenous levels. The BRCTs are not required, however, when rad9delBRCTs is overexpressed, as this confers a wild-type UV and arrest response. We conclude, therefore, that the BRCT domains act to increase the local concentration of Rad9 at sites of damage in G2/M by homodimerization, but are not required for arrest and repair of UV damage, per se.

Other data indicate that the BRCTs play a different role in response to MMS damage, which is considered to be S-phase specific. Disrupting dimerization of Rad9 through the BRCTs does not affect MMS resistance, though the BRCT domains are necessary for wild-type levels of viability in the presence of MMS. We expect that future research will tell us if Rad9 associates with other checkpoint or repair proteins during S-phase or can perhaps directly associate with DNA damage through the BRCT repeats.

We hope that knowledge of Rad9 functions mediated by the BRCT domains will illuminate Brca1 function – specifically, its contribution to cell cycle arrest, repair of DNA damage, and prevention of gross chromosomal alterations.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0127 supported this work.

RECOMBINATION PROTEINS AND THE POLκ ORTHOLOG, POL IV, ARE REQUIRED FOR STATIONARY-PHASE ANTIBIOTIC-RESISTANCE MUTATION

Joseph F. Petrosino, Liza D. Morales, Amanda R. Pendleton, and Susan M. Rosenberg

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

jp691032@bcm.tmc.edu

When cancers develop in tissues of non-dividing or slowly growing cells, the first cancerous cell must escape growth regulation and acquire mutations to become transformed. Mutational mechanisms specific to non-growing cells may facilitate this progression from quiescence to division. Similarly, when tumors develop resistance to chemotherapeutic drugs, such as mitotic inhibitors, they acquire mutations and other changes that allow growth in the presence of the drugs. Moreover, the growth stage when mutation is most active is not known. G_0, the analog of bacterial stationary phase, could be an important period for spontaneous mutation. To understand these processes, it is important to elucidate the mechanism(s) of mutation in non-dividing and slowly growing cells.

Frequently, bacterial resistance to antibiotics arises spontaneously as a result of mutation(s) at specific chromosomal loci. A clinically important example is resistance to ß-lactam antibiotics in enterobacterial pathogens other than *E. coli*. Loss-of-function mutations in the *ampD* gene, causing expression of the endogenous AmpC ß-lactamase, are found in resistant enterobacteria. ß-lactams kill actively dividing bacteria, and so stationary-phase, stress-induced mutation mechanisms might produce *ampD* mutations. *E. coli* carries all of the *amp* genes required for *ampC* expression except for *ampR*, the transcriptional activator of *ampC*. When the Enterobacter *ampRC* genes are placed on a plasmid in *E. coli*, ß-lactam resistance also arises *via ampD* mutation. We have integrated the Enterobacter *ampRC* genes into the *E. coli* chromosome to enable genetic dissection of possible roles of DNA metabolism proteins in stress-induced ß-lactam resistance-causing mutation of *ampD*. We find that *ampD* mutation rates in growing cells are not affected by mutations in recombination or SOS genes. However, ß-lactam resistant mutants arise in stationary phase, during starvation on lactose. Their formation requires the hRad51 homolog, RecA and the *E. coli* Polκ ortholog, Pol IV (DinB), a DNA damage (SOS)-induced protein. *ampD* mutant sequence analyses support the finding that a stress-promoted mutation mechanism is capable of producing ß-lactam resistance mutations in these cells.

The fact that human homologs of *E. coli* recombination and repair proteins have been implicated in breast cancer, hereditary nonpolyposis colon cancer (HNPCC), and other cancers makes this an important model system for mutational mechanisms relevant to oncogenesis, tumor progression, and resistance to chemotherapeutic agents.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0144 supported this work.

THE ROLE OF BE2/HSRBC IN DNA DAMAGE RESPONSE

Michael J. Peyton, Xie L. Xu, Kimberley E. Tomenga, Joseph Geradts, Sabine Zöchbauer-Müller, and John D. Minna

Hamon Center for Therapeutic Oncology Research,
University of Texas Southwestern Medical Center,
Dallas, TX 75390-8593

michael.peyton@utsouthwestern.edu

BE2/hSRBC was originally cloned due to its interaction with BRCA1 in a yeast two-hybrid assay and assumed to be a nuclear protein. Subsequent data showed that BE2/hSRBC localized to 11p15.4-15.5 a common site of LOH in breast cancer. It was shown to be down regulated in a variety of breast cancer cell lines typically by promoter hyper-methylation resulting in the loss of both mRNA and protein. Based on its location and its interaction with BRCA1, which plays a role in DNA repair, we are investigating the role of BE2/hSRBC in DNA repair.

Immunohistochemical staining was performed using an available monoclonal antibody. BE2/hSRBC was cloned into pEGFP-N3 (Clontech) using standard techniques. Polyclonal antibodies were produced using genetic immunization followed by peptide boosts and were tested by western blotting. BE2/hSRBC was transfected into breast cancer cells lacking its expression and tested in Comet assays as a measure of DNA repair activity.

Cell fractionation experiments localized BE2/hSRBC to the nucleus and membrane, while immunostaining localized it to the membrane. A BE2/hSRBC-GFP fusion protein appears to stain the entire cell. Since membrane fluorescence can appear cytoplasmic, current co-localization experiments with targeted color-variants are underway to resolve these possibilities. Polyclonal antibodies have been produced which recognize BE2/hSRBC protein on Western blots and are currently being tested in immunoprecipitation assays. Comet assays are currently unable to detect an effect of BE2/hSRBC on DNA repair. However, we are testing various parameters to improve this assay.

BE2/hSRBC localization may be dynamic with both membrane and nuclear compartmentation. Polyclonal antibodies have been produced and they are capable of detecting BE2/hSRBC on Western blots. At present we have no results to support the role of BE2/hSRBC in DNA repair. This suggests, perhaps, that its role is in signal transduction from the membrane to BRCA1.

The loss of BE2/hSRBC in breast cancer suggests its importance for diagnosis, while its interaction with BRCA1 suggests a potential role in therapy.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0422 and DAMD17-94-J-4077 supported this work.

ROLE OF DNA DOUBLE-STRAND BREAKS IN MUTATION IN GROWTH-INHIBITED CELLS

Rebecca G. Ponder and Susan M. Rosenberg

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

rp692236@bcm.tmc.edu

Although many traditional mutation assays focus on rapidly growing cells, many cancers arise in differentiated tissues in which cells are no longer actively dividing. We are studying a spontaneous mutation mechanism that occurs only in growth-inhibited cells of the model organism *Escherichia coli*. In the *E. coli* Lac assay, when Lac^- cells that carry a +1 frameshift mutation in a lactose gene located on an F' conjugative plasmid are plated on medium with lactose as the only carbon source, Lac^+ stationary-phase revertant colonies arise continuously over several days of starvation. We tested the postulate that the stationary-phase mutations result from acts of DNA double-strand break repair (DSBR). In one model for stationary-phase mutation, a DSBR intermediate primes DNA synthesis, during which pol IV, an error-prone polymerase required for stationary-phase Lac^+ mutation, is proposed to create errors that lead to mutation. One of the proteins required for both DSBR and Lac^+ stationary-phase mutation is RecA, a homologue of the human repair protein Rad51, which associates with the BRCA tumor suppressor proteins. F' plasmid transfer (Tra) proteins are also required; Tra functions induce single-strand nicks on the F', which could promote double-strand break (DSB) formation and subsequent mutation.

We report that specific DSBs introduced near *lac* on the F' sex plasmid activate stationary-phase mutation and substitute for Tra functions. To make specific DSBs, we constructed strains that express the double-strand endonuclease I-SceI and carry a single I-SceI cut site at one of four sites near *lac* on the F'. We find that introducing specific breaks on an F' that lacks Tra functions results in 50-2000-fold stimulation of Lac^+ stationary-phase mutation. Presence of both the I-SceI enzyme and cut site are required, as are recombination repair proteins and DNA pol IV. These results provide the first direct evidence that DNA DSBs can activate stationary-phase mutation and imply that the role of Tra functions is to promote the formation of DSBs. The introduction of DSBs activates a similar mechanism to that which produces Lac^+ stationary-phase mutation, and not an alternative pathway, because recombination proteins and DNA pol IV are required. The mechanisms that promote stationary-phase mutation in *E. coli* may lead to genomic instability in all organisms and, in humans, may contribute to cancer formation and tumor progression, as well as to the development of chemotherapeutic-resistant tumors.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0145 supported this work.

TEMPERATURE MODULATES THE HIGHER-ORDERED SELF-ASSOCIATION OF RAD52

Wasantha Ranatunga, Doba Jackson, and Gloria E. O. Borgstahl

Department of Chemistry, The University of Toledo,
2801 West Bancroft Street, Toledo, OH 43606

wranatunga@hotmail.com

Defects in recombination-based DNA repair lead to human breast cancer and familial degenerative diseases. The RAD52 epistasis gene products, especially the human RAD52 protein plays important role in double-strand break (DSB) repair. hRAD52 has shown to be interacting with many proteins in recombination-based DSBs repair pathway. The focus of this work is to further understanding of the molecular basis of DSBs by solving the three-dimensional structure of hRAD52. The hRAD52 forms ring structure in solution and multiple level of aggregation of rings.

Due to the biological interest of hRAD52 and the apparent biochemical importance of RAD52 self-association in DNA-repair, we studied its multiple levels of self-association and stability using biophysical methods such as dynamic light scattering (DLS) and differential scanning calorimetry (DSC). The stability of wild-type RAD52 was studied by DSC. To investigate the basis for the extreme stability of RAD52 that was discovered, two mutants were also studied, RAD52 (1-192) and RAD52 (218-418). The effects of temperature and protein concentration on the hydrodynamic radius (RH) of RAD52 were studied by DLS.

We found that the aggregation is due to two levels of self-association of hRAD52, ring formation and association of rings with rings. DSC profiles and DLS data indicated that hRAD52 protein is extremely stable and multiple levels of self-association of hRAD52 can be disrupted by heating up to 50 °C.

A hypothetical model of the effects of protein aggregation state on thermal stability was developed. Based on these findings, a novel approach for purification of hRAD52 and for crystallization was established. This research will contribute a detailed understanding of the molecular mechanisms of breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0469 supported this work.

CHECKPOINT PATHWAYS AS THERAPEUTIC TARGETS FOR BREAST CANCER

Honican D.M., Kecheng J., Chen Y., Searle J.S., Schollaert K., and Sanchez Y.

University of Cincinnati, Department of Molecular Genetics, Biochemistry, and Microbiology

yolanda.sanchez@uc.edu

Checkpoints are biochemical pathways that provide cells with a mechanism to detect DNA damage, and respond by arresting the cell cycle to allow DNA repair. The inability to respond to such damage leads to increased genomic instability, which can contribute to deregulation of cellular growth and cancer. Mutation in mammalian genes such as p53, BRCA1 and ATM, which abrogate this response, cause a genetic predisposition to cancer. The ATM pathway and tis role in checkpoint control are conserved among eukaryotes. In the yeasts, the Atm-like proteins rad3 and Mec1 function upstream of the DNA damage-inducible Chk1 (checkpoint kinase 1). We have shown that human and budding yeast Chk1 have a role in preventing mitosis following DNA damage. The long term goals of these studies are to 1) use the mammalian culture system to determine whether inactivation of the Chk1 pathway is a feasable approach to breast cancer therapy, and 2) determine whether the same genetic factors that make Chk1 essential in order to survive oxidative stress also affect individual susceptibility to low dose radiation. For this we are carrying a two pronged approach with two model systems: mammalian and yeast. Using Biochemical approaches we have uncovered a physical and regulatory interaction between Chk1 and the DNA-PK protein complexes, which regulate the repair of double strand breaks. Chk1 stimulates DNA binding of DNA-PK complexes, and this leads to enhanced kinase activity of DNA-PK. By understanding the connection between the checkpoint pathways and DNA repair we will establish targets for drug discovery.

We have also determined that yeast Chk1 becomes activated in response to oxidative stress. We are using genetic approaches to identify mutations that render cells dependent on Chk1 for viability in response to oxidative stress. We will determine the role of these components in the response to ionizing radiation. These results will allow us to evaluate and understand both the therapeutic effects and the individual risk of exposure to radiation and could allow us to maximize the efficacy of our therapeutic approaches to cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0203 supported this work.

INTERACTION OF THE ACIDIC DOMAIN OF PIAS1 WITH P53, RAD51, AND RAD52

David Schild

Life Sciences Division,
Lawrence Berkeley National Laboratory

dschild@lbl.gov

Characterization of BRCA1 and BRCA2 has strongly implicated homologous recombinational repair (HRR) as a pathway important in breast cancer. Rad51 and Rad52 are two additional proteins important for HRR, and we are characterizing some of their interactions. Using the yeast two-hybrid system, human proteins PIAS1 and PIAS3 (protein inhibitors of activated STATs) have been found to specifically interact with both the human Rad51 and Rad52 proteins. Neither PIAS1 nor PIAS3 interacts with any of the five human proteins distantly related to Rad51, showing that these interactions are specific. Recently, PIAS1 has been shown by others to interact with p53 and to be involved in its sumoylation (Kahyo, et al., 2001). Sumoylation is related to ubiquitination, but does not appear to tag a protein for degradation. PIAS1 and PIAS3 appear to be SUMO ligases.

The interaction between PIAS1 and hRad51 has been confirmed using purified hRad51 and PIAS1-GST fusion proteins. The acidic domain of PIAS1 has been shown to interact with hRad51, hRad52 and p53. The interacting region has been further narrowed to 21 amino acids, and individual residues are currently being mutated to determine which mediate these interactions. We are currently examining the regions of hRad51 and hRad52 that participate in this interaction. A working hypothesis is that the acidic domain of PIAS1 may bind to the DNA-binding domain of proteins with which it interacts. Additional work is needed to determine if PIAS1 in general, and its acidic domain in particular, interacts with other sumoylated repair proteins, such as WRN, BLM, TOPO I and TOPO II.

A goal is to isolate peptides that specifically interact with only a single DNA repair protein. Such peptides would be useful to better characterize these proteins. If the acidic domain of PIAS1 interacts with the DNA-binding domain of its partners, then these peptides would also be useful in characterizing DNA-binding domains. For example, peptides that interact specifically with the DNA-binding domain of p53 might be useful in disrupting this aspect of p53 activity, without disrupting its other activities. If a DNA-binding domain of a PIAS1-interacting protein has not been characterized, these experiments might be used to map its location.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0545 supported this work.

C-MYC ALTERS DNA DAMAGE-INDUCED CELL CYCLE CHECKPOINTS IN HUMAN MAMMARY EPITHELIAL CELLS

Joon-Ho Sheen and Robert B. Dickson

Department of Oncology and Lombardi Cancer Center
Georgetown University Medical Center
Washington, DC 20007

sheenj@georgetown.edu

Study of the mechanism(s) of genomic instability induced by c-myc proto-oncogene has the potential to shed new light on its well-known oncogenic activity. However, an underlying mechanism(s) for this phenotype is largely unknown. In the current study, we investigated the effects of c-Myc overexpression on the DNA damage-induced G1/S checkpoint, in order to obtain mechanistic insights on how deregulated c-Myc destabilizes the cellular genome. The DNA damage-induced checkpoints are among the primary safeguarding mechanisms for genomic stability, and alterations of cell cycle checkpoints are known to be crucial prerequisites for certain types of genomic instability, such as gene amplification. The effects of c-Myc overexpression were studied in human mammary epithelial cells (HMEC), as one approach to understand the c-Myc-induced genomic instability, in the context of mammary tumorigenesis. Inappropriate entry into S phase was then confirmed using a BrdU incorporation assay, measuring de novo DNA synthesis, following IR. A direct involvement of c-Myc overexpression in alteration of the G1/S checkpoint was then confirmed by utilizing the MycER™ construct, a regulatable c-Myc. A transient excess of c-Myc activity, provided by the activated MycER™, was similarly able to induce the inappropriate de novo DNA synthesis, following IR. Significantly, the transient expression of full-length c-Myc in normal mortal HMECs also facilitated entry into S phase and the inappropriate de novo DNA synthesis, following IR. We observed inappropriate hyperphosphorylation of Rb and then the reappearance of cyclin A, following IR, selectively in the full-length c-Myc-overexpressing MCF10A cells. Based on these results, we propose that c-Myc attenuates a safeguard mechanism for genomic stability. The genomic instability is a driving force for tumor progression. Therefore, elucidation of c-Myc-induced genomic instability in the mammary cell will facilitate the understanding about the c-Myc-induced mammary tumorigenesis. This would also help finding a novel target for the prevention of gene amplification phenotype and proliferation of tumor cells with the phenotype.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9205 and DAMD17-00-1-0270 supported this work.

FUNCTIONAL CHARACTERIZATION OF BCCIP, A BRCA2-INTERACTING PROTEIN

Xiangbing Meng, Xu Guo, Jingmei Liu, and Zhiyuan Shen

Department of Molecular Genetics and Microbiology, University of New Mexico School of Medicine, 915 Camino de Salud, NE, Albuquerque, NM 87131

zshen@salud.unm.edu

The breast cancer gene BRCA2 plays critical roles cellular response to DNA damage. A newly identified protein, designated BCCIP for BRCA2 and CDKN1A (p21) interacting protein, was found to interact with a highly conserved BRCA2 region coded by exons 14-24 (Liu, J., Yuan, Y., Huan, J., and Shen, Z. 2001. Oncogene, 20:336-35.). Based on comparison of human BCCIP with homologues from other species, three distinguishable domains can be identified in the BCCIP protein family: a N-Terminal Acidic Domain (NAD), an Internal Conserved Domain (ICD) that is highly conserved throughout all species, and a C-terminal Variable Domain (CVD). Developing evidence suggest that BCCIP plays an important role in cell growth and DNA damage response.

In this study, we have mapped the BRCA2-binding domain of BCCIP to an ICD region using a series of BCCIP fragments in immunoprecipitation. We have found that the RAD51 protein co-precipitates with the same domain of BCCIP that interacts with BRCA2, suggesting the co-existence of RAD51 in the BRCA2-BCCIP complex. Furthermore, using a site-specific DNA double strand break (DSB) induced homologous recombination assay, we have found that expression of the BRCA2-interacting domain of BCCIP inhibits DSB-induced homologous recombination. These results suggest an important role of the BRCA2/BCCIP interaction in DSB-induced homologous recombination.

It was reported that BRCA2 protein co-localizes with PCNA in response to replication blockage. We have also identified an interaction between BCCIP and the PCNA by in vitro protein binding assays and co-immunoprecipitation of endogenous BCCIP with PCNA. These results further suggest a role of BCCIP in the same cellular pathways as BRCA2.

Finally, we have also found over-expression of BCCIP inhibit the growth of breast cancer cell MCF7, but not non-transformed fibroblasts. The mechanism of this inhibition is being investigated.

Original work supported by the U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8198.

PHENOTYPE-BASED IDENTIFICATION OF MOUSE CHROMOSOME INSTABILITY MUTANTS

Naoko Shima, Suzanne Hartford, Ted Duffy, Lawriston Wilson, Kerry Schimenti, and John Schimenti

The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609

nshima@jax.org

Increasing evidence indicates that defects in DNA double strand break (DSB) repair can cause chromosome instability, resulting in cancer. Involvement of the breast cancer susceptibility gene products BRCA1 and BRCA2 in DSB repair, particularly by homologous recombination, highlights the importance of DSB repair in genome maintenance and breast tumor suppression. Moreover, elevated radiosensitivity of lymphocytes from unselected breast cancer patients has been reported, implicating potential inactivation of DSB repair during breast tumorigenesis. Because *BRCA1* and *BRCA2* cannot account for all familial breast cancer, it has been suggested that there might be other susceptibility genes. We hypothesize that there are unknown genes in DSB repair that might affect breast cancer risk.

To identify new mammalian genes that are involved in genome maintenance via DSB repair, we have conducted a screen for mouse chromosome instability mutants using a highly sensitive and reproducible flow cytometric peripheral blood micronucleus assay. Micronuclei result from chromosome break/loss and are extensively used for quantitative analysis of *in vivo* chromosome damage. Micronuclei in mouse erythrocytes are clearly enumerated, because erythroblasts expel their nuclei, but not micronuclei, after their last mitotic division.

Among 422 offspring derived from males mutagenized with a potent germline mutagen *N*-ethyl-*N*-nitrosourea, one recessive mutation conferring spontaneous chromosome instability and radiation sensitivity was identified. This mutation *chaos1* (chromosome aberration occurring spontaneously 1) was genetically mapped to a 1.3 Mb interval on Chromosome 16 that contains *Polq*, the mouse ortholog of the human *POLQ* encoding DNA polymerase theta. *Polq* is homologous to the *Drosophila mus308* gene essential for DNA inter-strand cross-link repair by homologous recombination; nevertheless its function is unknown in mammals. *chaos1/chaos1* mice were found to be sensitive to a cross-linking agent mitomycinC by the micronucleus assay and contained a non-conservative mutation in *Polq*, making it a strong candidate for *chaos1*. Experiments such as rescue of the mutant phenotype with a bacterial artificial chromosome, or non-complementation with a targeted allele of *Polq* are currently underway to confirm whether a mutant allele of *Polq* causes *chaos1*phenotype.

The goal of this study is to investigate the effects of identified mutation(s) on DSB repair and cancer development in whole animals, creating animal models for cancer studies. We believe that the generation of mouse models will be useful in the identification and testing of drugs designed to combat breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0277 supported this work.

TRANSDUCTION OF EXONUCLEASE III INTO MITOCHONDRIA OF BREAST CANCER CELLS AND ITS EFFECT ON MITOCHONDRIAL DNA REPAIR

Inna N. Shokolenko, Mikhail F. Alexeyev, Susan P. LeDoux, and Glenn L. Wilson

University of South Alabama College of Medicine, Mobile, AL 36688

inna_shokolenko@hotmail.com

Previous results from our lab demonstrated that breast cancer cells stably transfected with E. coli Exonuclease III (ExoIII) targeted to mitochondria, have diminished mtDNA repair capacity and show decreased long-term cell survival following oxidative stress. Because of the temporary nature of cancer therapy, only transient introduction of proteins into cells is required. We employed a novel method of protein transduction to deliver proteins, bearing the TAT protein transduction domain from HIV virus and the mitochondrial targeting signal (MTS) from manganese superoxide dismutase, into mitochondria of breast cancer cells. Initial experiments with Green Fluorescent Protein (GFP), containing the TAT domain and MTS, confirmed the transduction and mitochondrial localization of GFP in breast cancer cell line MDA-MB-231. Subsequent work resulted in expression and purification of catalytically active ExoIII, containing the TAT domain and MTS. Transduction of MTS-ExoIII-TAT protein in MDA-MB-231 cells resulted in its mitochondrial accumulation. Preliminary data from DNA repair experiments indicate that transduction of ExoIII into mitochondria leads to decrease in mtDNA repair capacity following oxidative stress. Studies are under way to determine the stability of transducted protein in the cells and the effect of its presence on a long-term cell survival.

The interference with the normal mtDNA repair process, caused by the direct delivery of specific protein into mitochondria, results in the sensitizing cells to oxidative damage. The ability to sensitize cancer cells to therapy involving oxidative DNA damage, such as radiation therapy, would be highly beneficial for successful cancer treatment because it could lead to a better killing effect that, at the same time, could be achieved with the lower doses, resulting in less severe side effects.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0658 supported this work.

THE HUMAN RAD51B-RAD51C COMPLEX ACTS AS A MEDIATOR OF RAD51/RPA-CATALYZED DNA STRAND EXCHANGE

Stefan Sigurdsson,[1] Stephen Van Komen,[1] Wendy Bussen,[1] David Schild,[2] Joanna S. Albala,[3] and Patrick Sung[1]

[1]Department of Molecular Medicine/Institute of Biotechnology, University of Texas Health Science Center at San Antonio; [2]Life Science Division, Lawrence Berkeley National Laboratory; [3]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory

sigurdsson@uthscsa.edu

Studies in Saccharomyces cerevisiae have identified a number of genes required for meiotic and mitotic recombination. These genes, collectively referred to as the RAD52 epistasis group, are involved in the repair of DNA double strand breaks through a recombination mechanism. Double strand breaks can be induced either by exogenous agents like ionizing radiation or formed during replication of a damaged DNA template. Genetic and biochemical studies have suggested that the structure and function of the RAD52 epistasis group genes are highly conserved from yeast to humans. Interestingly, the efficiency of DNA double strand break repair in mammals is dependent on the tumor suppressors BRCA1 and BRCA2. This underscores the importance of studying the mechanistic basis of the recombination machinery. The human Rad51 protein is a functional homolog of Escherichia coli RecA protein, and like RecA, it can catalyze homologous DNA pairing and strand exchange reactions. To do so, the Rad51 protein must first assemble onto the single stranded DNA as a nucleoprotein filament, but this assembly is strongly inhibited by secondary structure in the single stranded DNA template. The secondary structures are removed by the single strand DNA binding protein RPA, which is essential for the efficiency of the homologous DNA pairing and strand exchange reaction. Interestingly, RPA can also compete with Rad51 for binding sites on the DNA, which can suppress the pairing and strand exchange activity of Rad51. Various proteins, called recombination mediators, are able to overcome the suppressive nature of the single strand binding proteins in prokaryotes and yeast cells. In yeast, two mediators, Rad52 and the Rad55/Rad57 complex, have been characterized. Interestingly five Rad55/Rad57 like proteins, called Rad51 paralogs, have been identified in human cells. We show that two of those Rad51 paralogs, Rad51B and Rad51C, are associated in a stable complex in human cells. The complex was purified to near homogeneity and was found to possess a single-stranded DNA stimulated ATPase activity. The Rad51B-Rad51C complex binds single-stranded DNA and promotes Rad51 mediated homologous pairing and strand exchange under conditions where Rad51 must compete with RPA for binding sites on the ssDNA template.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0412 supported this work.

BRCA2 AND SENSITIVITY OF CAPAN-1 CELL LINE TO DNA-DAMAGING AGENTS

Li-Kuo Su

The University of Texas M.D. Anderson Cancer Center

lsu@mdanderson.org

People carrying a mutant allele of BRCA2 have increased risk for breast, ovarian, pancreatic and other types of cancer. BRCA2 has been shown to interact with RAD51, which is an evolutionary conserved protein important for repairing DNA break (DSB). Mouse cells deficient of Brca2 are highly sensitive to DNA damaging agents and have aberrant chromosomes. In addition, Capan-1 cell line, which is a human pancreatic cancer cell line and is the only human cell line known to not express wild-type BRCA2, has been shown to be deficient in repairing DSB and be more sensitive to DNA damaging agents than did other human cell lines. These observations have led to the suggestion that BRCA2 plays an important role in repairing double strand DSB. The increased sensitivity of Capan-1 to DNA damage has been attributed to its lack of wild-type BRCA2.

I have investigated whether expression of the wild-type BRCA2 is sufficient to reduce the sensitivity of Capan-1 cells to DSB. I have generated two Capan-1 derivatives that constitutively express the wild-type BRCA2 and two that express the wild-type BRCA2 under the regulation of tetracycline. The sensitivity to gamma-radiation of the two constitutive BRCA2-expressing Capan-1 derivatives and that of the parental Capan-1 cells were found to have no detectable difference. When Capan-1 derivatives that expressed the wild-type BRCA2 under the regulation of tetracycline were investigated, there was again no detectable difference in their sensitivity to gamma-radiation between they did and did not express the wild-type BRCA2. The sensitivity of these cells to DNA damaging chemicals was also found to be no difference between cells expressing and not expressing the wild-type BRCA2. These results suggest that expression of the wild-type BRCA2 alone is not sufficient to alter the sensitivity of Capan-1 cells to DSB. BRCA2 has been shown to be important for RAD51 to form nuclear foci in cells after gamma-radiation becasue Capan-1 cells are defect in this activity. Our investigation showed that expression of the wild type BRCA2 did not restore the ability of Capan-1 cells to form RAD51 nuclear foci after gamma-radiation. This could explain why expression of the wild-type BRCA2 does not alter the sensitivity of Capan-1 cells to DNA damaging agents.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0317 supported this work.

FUNCTIONAL INTERACTIONS BETWEEN RECOMBINANT FACTORS RAD51 AND RAD54 IN EFFICIENT HETERODUPLEX DNA JOINT FORMATION

Stephen Van Komen, Stefan Sigurdsson, Galina Petukhova, and Patrick Sung

Institute of Biotechnology and Department of Molecular Medicine, University of Texas Health Science Center at San Antonio, TX

vankomen@uthscsa.

Rad51 and Rad54, members of the RAD52 epistasis group, are key recombination factors responsible for the accurate repair of DNA double-strand breaks and genetic recombination. These proteins are highly conserved in both structure and function among eukaryotes, from yeast to humans. Rad51, the eukaryotic equivalent of E. coli RecA, is central to recombination processes by virtue of its ability to mediate the homologous DNA pairing and strand exchange reaction that yields heteroduplex DNA. Rad54, a member of the Swi2/Snf2 family of proteins, possesses a robust dsDNA-dependent ATPase activity. Rad54 and Rad51 interact and inclusion of catalytic amounts of Rad54 to a homologous DNA pairing and strand exchange reaction dramatically stimulates the Rad51-mediated pairing rate and renders D-loop formation by Rad51 very efficient.

Upon ATP hydrolysis, Rad54 generates unconstrained negative and positive supercoils on the template DNA which likely result from a tracking motion of Rad54 on the template with positive supercoils proceeding and negative supercoils trailing the Rad54. This DNA topological remodeling by Rad54 is greatly stimulated by Rad51. As Rad54 tracks, or threads the incoming DNA through its fold, the already associated Rad51-ssDNA nucleoprotein complex samples the duplex for homology at a higher rate. Furthermore, as Rad54 tracks on DNA, the negative supercoils produced lead to a transient DNA strand separation that marks the duplex DNA highly sensitive to the single-strand specific P1 nuclease. This strand-opening is expected to promote DNA joint formation by Rad51.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0414 supported this work.

BRCA1 FACILITATES MICROHOMOLOGY-MEDIATED END-JOINING OF DNA BREAKS

Qing Zhong, Chi-Fen Chen, Phang-Lang Chen, and Wen-Hwa Lee

Department of Molecular Medicine/Institute of Biotechnology, The University of Texas Health Science Center at San Antonio, TX

leew@uthscsa.edu

BRCA1 is critical for the maintenance of genomic stability, in part, through its interaction with the Rad50/Mre11/Nbs1 complex, which occupies a central role in DNA double-strand break (DSB) repair mediated by non-homologous end-joining (NHEJ) and homologous recombination (HR). BRCA1 has been shown to be required for homology-directed recombination repair. However, the role of BRCA1 in NHEJ, a critical pathway for the repair of DSBs and genome stability in mammalian cells, remains elusive. Here, we established a pair of mouse embryonic fibroblasts (MEFs) derived from 9.5 day old embryos with genotypes Brca1+/+:p53-/- or Brca1-/-:p53-/-. The Brca1-/-:p53-/- MEFs appear to be extremely sensitive to ionizing radiation. The contribution of BRCA1 in NHEJ was evaluated in these cells using three assay systems. Firstly, transfection of a linearized plasmid in which expression of the reporter gene required precise end-joining indicated that Brca1-/- MEFs display a moderate deficiency as compared to Brca1+/+ cells. Secondly, using a retrovirus infection assay dependent on NHEJ, a 5-10 fold reduction in retroviral integration efficiency in Brca1-/- cells was observed as compared to the Brca1+/+ MEFs. Thirdly, Brca1-/- MEFs exhibited a 50-100 fold deficiency in micro-homology mediated end-joining activity of a defined chromosomal DNA double-strand break introduced by a rare-cutting endonuclease I-SceI. These results provide evidence that Brca1 has an essential role in micro-homology mediated end joining, and suggest a novel molecular basis for its caretaker role in the maintenance of genome integrity.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0457 supported this work.

CELL-FREE AND CELLULAR ACTIVITIES OF SEQUENCE-SELECTIVE POLYAMIDE-BASED DNA ALKYLATING AGENTS

Yong-Dong Wang,[1] Jaroslaw Dziegielewski,[1] Aileen Y. Chang,[2] Peter B. Dervan,[2] and Terry A. Beerman[1]

[1]Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute; [2]Division of Chemistry Engineering, California Institute of Technology

gnodgnoy@yahoo.com

DNA alkylating agents represent a class of anti-tumor drugs whose members include some of the most useful clinical agents. This group of agents are generally highly reactive with DNA but demonstrate limited DNA sequence selectivity. In contrast, pyrrole-imidazole polyamides are synthetic ligands that recognize specific DNA sequences with high affinities. Our previous study has shown that hairpin polyamides have the potential to effect gene transcription by specifically targeting transcription promoters, such as Her2/neu promoter. However, these reversible DNA binding agents lack the ability to damage DNA and there have been few reports to date that directly demonstrate specific down-regulation of endogenous gene expression under cellular conditions nor that polyamides can inhibit cell growth.

In an effort to combine the properties of polyamides and DNA alkylating agents, we synthesized an eight-ring hairpin polyamide conjugated to an alkylating moiety related to CC-1065 (a classic alkylating agent). This conjugated agent, which covalently attaches to specific sequences in the minor groove of DNA, has previously been shown to alkylate a single adenine flanking the polyamide-binding site on a 277-bp DNA fragment under cell-free conditions. In this study, we tested this hybrid molecule for its ability to interact with a higher order eukaryotic DNA structure (SV40 viral DNA). Our results indicate that the hybrid compound effectively targets the polyamide-binding sequence, introduces DNA strand damage to naked and chromosomal DNA and inhibits DNA replication under both cell-free and cellular conditions. In addition, cell growth inhibition and cell cycle arrest were observed.

In summary, we synthesized a hybrid compound of a polyamide and an alkylating agent. Characteristics of both parent compounds are maintained in this novel hybrid agent. This study demonstrates for the first time that a DNA alkylating agent has an ability to target cellular DNA at a predetermined sequence, which represents a new type of DNA damaging agents that have the potential to target specific breast cancer genes.

Original work supported by the U.S. Army Medical Research and Materiel Command under DAMD17-97-1-7064 and current work supported by the NCI.

53BP1 IS A COMPONENT OF THE SAME GENOTOXIC STRESS-RESPONSIVE PATHWAY–BRCA1 IS INVOLVED

Irene Ward, Ph.D., and Junjie Chen, Ph.D.

Mayo Clinic, Rochester, MN 55905

rappold.irene@mayo.edu

53BP1 has been reported to interact with the central DNA-binding domain of the tumor suppressor p53 and to enhance p53-dependent transcription. The C-terminus of 53BP1 contains two BRCT (BRCA1-C-terminus) motifs. This motif was first identified in the C-terminal region of the product of the breast cancer susceptibility gene 1 (BRCA1) and has since been found in a large number of proteins involved in various aspects of cell cycle control, recombination and DNA repair. We therefore asked whether 53BP1 is –like BRCA1- involved in the maintenance of genomic integrity.

To address this question we generated antibodies against 53BP1 and performed co-immunofluorescence and co-immunoprecipitation analyses in the presence or absence of genotoxic agents.

We could show that 53BP1 becomes hyperphosphorylated and forms discrete nuclear foci at the sites of DNA lesions in response to ionizing radiation. These foci colocalize with BRCA1 foci several hours after DNA damage. Furthermore, small amounts of 53BP1 could be co-immunoprecipitated with BRCA1 after irradiation but not in untreated cells. Like BRCA1, 53BP1 is phosphorylated by ATM (ataxia telangiectasia mutated) *in vivo* following ionizing radiation. Both proteins were also found to localize to stalled replication forks in response to replicational stress.

We conclude that 53BP1 is involved in the early response to genotoxic stress. Given its interactions with p53 and BRCA1 we speculate that 53BP1 might also act as a tumor suppressor.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0317 supported this work.

SOMATIC MITOCHONDRIAL DNA MUTATIONS IN BREAST CANCER

Lee-Jun C. Wong, Ph.D., and Duan-Jun Tan, Ph.D.

Department of Oncology, Georgetown University Medical Center, Washington, DC 20007-2113

wonglj@georgetown.edu

The major function of mitochondria is to generate energy in the form of ATP through oxidative phosphorylation pathway. The mutation rate of mitochondrial DNA (mtDNA) is at least 10 times higher than that of nuclear DNA due to the lack the protective histone proteins and the limited DNA repair mechanisms. In addition, mitochondrion is the major site of reactive oxygen species (ROS) production, thus, is susceptible to oxidative DNA damage which is believed to be associated with cancer. The important roles of mitochondria in energy metabolism, the generation of ROS, aging, and the initiation of apoptosis have suggested that mitochondria may serve as the switching point between cell death and abnormal cell growth, thus contributing to the neoplastic process. The purpose of this study is to comprehensively scan for mtDNA mutations in breast cancer using an effective method such that the mtDNA mutation spectrum can be determined and their importance in breast cancer can be studied.

Thirty-two pairs of overlapping primers were used to amplify the entire 16.6 kb mitochondrial genome. Temporal temperature gradient gel electrophoresis (TTGE) was used to detect mutations followed by sequencing of the DNA fragments that show differences in banding patterns between normal and tumor. The results of DNA sequence analysis were compared with the published Cambridge sequence.

A total of 27 somatic mtDNA mutations were found in 14 out of 19 tumors (74% positive). Twenty-two mutations are in the hypervariable D loop region (81.5%), 4 in mRNA, and 1 in rRNA region. Six tumors had 1 mutation, and each of the remaining tumors had multiple somatic mtDNA mutations. In addition, 102 germline mtDNA variations were found. MtDNA deletions were not detected in breast tumor. Evaluation of somatic instability at 11 microsatellite regions revealed that insertion and deletion occurred only in the D loop region of np 303-309 where a stretch of 7 Cs was located. Microsatellite instability (MSI) was not detected in any of the other regions, suggesting that the 303-309 is probably a mutation hot spot rather than the refection of a true MSI.

In conclusion, somatic mtDNA mutation is a general phenomenon in tumors, including breast cancer. Mutations anywhere in mitochondrial genome may affect the efficiency of mtDNA replication, transcription, or translation. Mutations in the coding region may have significant functional effect so that the mutant mitochondria acquire growth advantage in tumor cells. Because of the high copy number of mtDNA, identification of somatic mtDNA mutations and their pathogenic consequences in breast cancer offers an excellent molecular marker for earlier detection and prognosis. The results will also allow us to evaluate if mitochondria DNA can be a potential therapeutic target for cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0258 supported this work.

DISSECTING THE DNA SEQUENCES ENCODING THE POLYMORPHIC POLYGLUTAMINE TRACT OF AIB1 GENE

Lee-Jun C. Wong, Ph.D., and Pu Dai, Ph.D.

Department of Oncology, Georgetown University Medical Center, Washington, DC 20007-2113

wonglj@georgetown.edu

The amplified in breast cancer (AIB1) gene 1 belongs to a family of nuclear receptor co-activators that bind to nuclear hormone receptors and stimulate the hormone dependent transcription activation. Similar to androgen receptor (AR), the AIB1 gene contains a polymorphic polyglutamine (poly Q) tract. The shorter poly Q repeat in AR has been shown to be associated with more aggressive prostate cancer. Haiman et al., found that poly Q repeat genotype did not influence postmenopausal breast cancer risk among Caucasian women in the general population. However, a matched case-control study of BRCA1 and BRCA 2 mutation carriers found that women with at least 28 poly Q repeats in AIB1 gene had higher breast cancer risk when compared to women who carried alleles with fewer poly Q repeat. These studies measured the repeat by fragment length analysis only. The purpose of this study is to analyze the actual DNA sequences encoding the poly Q repeat such that the sequence characteristic, in addition to the length polymorphism, can be correlated with breast cancer risk.

A total of 107 DNA samples from 16 breast cancer cell lines, 32 primary breast tumors, 16 blood samples from carriers of BRCA1/BRCA2 mutation, and 43 blood specimens from normal individuals in general population, were studied. The poly Q encoding DNA fragment was PCR amplified followed by cloning into TOP10 vector. At least 6 clones from each sample were sequenced on ABI377 sequencer. Amplification of AIB1 gene was measured by real-time PCR.

Twenty-three distinct poly Q encoding sequence patterns were found. Statistical analysis showed that a significantly higher proportion of breast tumors or cancer cell lines contained rare sequence patterns when compared to the general population. The proportion of women having at least one allele with less than 28 repeats in the sporadic breast cancer group is significantly higher than that in BRCA1/BRCA2 mutation carriers or the general population. Two rare sequences, a short 17-repeat and a CAG to CGG (gln to arg) mutation were found. These alterations may have significant effect on the function of AIB1. The poly Q encoding sequences did not seem to correlate with the AIB1 gene amplification.

Our results demonstrate that the encoding sequence of poly Q of AIB1 gene is unstable in breast cancer cell lines and primary tumors, not so much by large expansion or reduction of the poly Q repeat, but by point mutations, small deletion or insertion of 1 or 2 triplets. Both sequence heterogeneity in the poly Q encoding region and the degree of gene amplification may contribute to the role of AIB1 gene in the tumorigenesis of breast cancer. This study reveals the importance of qualitative and quantitative changes of AIB1 in modifying an individual's breast cancer risk.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0257 supported this work.

CHK2 ACTIVATION AND PHOSPHORYLATION-DEPENDENT OLIGOMERIZATION

Xingzhi Xu, Lyuben M. Tsvetkov, and David F. Stern

Department of Pathology,
Yale University, New Haven, CT 06510

xingzhi.xu@yale.edu

When DNA is damaged, cells activate checkpoint pathways that halt the cell cycle and induce the transcription of genes that facilitate repair. Checkpoint defects may result in genomic instability, a mutagenic condition that predisposes organisms to cancer. On the other hand, DNA damaging agents, in the form of gamma irradiation and chemotherapeutic drugs are the mainstays of most current cancer treatment regimens. Proper manipulation of checkpoint genes may benefit chemo- and radiotherapy.

The tumor suppressor gene CHK2 encodes a versatile effector serine/threonine kinase involved in responses to DNA damage. Mutations in the CHK2 gene have been demonstrated in a variety of tumors including breast cancer. Chk2 has an amino-terminal SQ/TQ cluster domain (SCD), followed by a forkhead-associated (FHA) domain and a carboxyl-terminal kinase catalytic domain. Mutations in the SCD or FHA domain impair Chk2 checkpoint function. We show here that autophosphorylation of Chk2 produced in a cell-free system requires trans-phosphorylation by a wortmannin-sensitive kinase, probably ATM or ATR. Both SQ/TQ sites and non-SQ/TQ sites within the Chk2 SCD can be phosphorylated by active Chk2. Amino acid substitutions in the SCD and the FHA domain impair auto- and trans-kinase activities of Chk2. Chk2 forms oligomers that minimally require the FHA domain of one Chk2 molecule and the SCD within another Chk2 molecule. Chk2 oligomerization in vivo increases after DNA damage, and, when damage is induced by gamma irradiation, this increase requires ATM. Chk2 oligomerization is phosphorylation-dependent, and can occur in the absence of other eukaryotic proteins. Chk2 can cross-phosphorylate another Chk2 molecule in an oligomeric complex. Induced oligomerization of a Chk2 chimera in vivo concomitant with limited DNA damage augments Chk2 kinase activity. These results suggest that Chk2 oligomerization regulates Chk2 activation, signal amplification, and transduction in DNA damage checkpoint pathways.

Our results may provide some mechanistic information for manipulating Chk2 activation and ultimaely benefit breast cancer prevention and treatment.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0465 supported this work.

OXIDASES AS BREAST CANCER ONCOGENES: A TRANSGENIC APPROACH TO ESTABLISH NEOPLASTIC TRANSFORMATION

Sailesh Surapureddi, Yuzhia Jia, Ritu Nayar, Janardan K. Reddy, and Anjana V. Yeldandi

Department of Pathology, Northwestern University Medical School, Chicago, IL 60611

a-yeldandi@northwestern.edu

Carcinoma of the breast is overwhelmingly a disease of females. The established risk factors are both non-hormone mediated and hormone-mediated. Oxygen free radicals are a well-established risk factor for cancer and aging. Evidence for the role of free radical-induced DNA damage in aging, and cancer comes from the correlation between high consumption of fruit and vegetables, or of specific dietary antioxidants and a relatively low incidence of several types of cancers. Free radical induced injury is known to aid in the development of breast cancer. The idea of free radical induced injury having a role in breast cancer development is intriguing since it opens up the possibility of antioxidants being able to prevent its development.

Our hypothesis is that the increased incidence of breast cancer in the United States is due to increased generation of reactive oxygen species (ROS) in the breast epithelium during the reproductive period and antioxidant activity will be beneficial in preventing breast cancer. Xanthine oxidase converts xanthine to uric acid and urate oxidase (UOX) hydrolyzes uric acid in lower mammals to allantoin, an end product of purine metabolism and in the process generates H2O2 and free radicals into the surrounding milieu. These free radicals are responsible for DNA damage and ultimately cell injury. To assess if urate oxidase over-expression in breast epithelium leads to neoplastic transformation, we have employed in vitro and in vivo transgenic approaches. Previously we have shown that UOX when stably expressed in cell lines, transform and develop tumors in nude mice. To study the role of these oxidases in vivo, we have established a transgenic model, to express UOX in mammary tissue under the control of MMTV-LTR promoter. Our model system helps to test the role of reactive oxygen species in causing cell death, cell proliferation and neo plastic transformation in mammary cells expressing urate oxidase.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9336 supported this work.

BASE EXCISION REPAIR GENE MUTATIONS AND POLYMORPHISMS AS A POTENTIAL MODIFIER OF BREAST CANCER RISK

Anthony T. Yeung,[1] Travis Burleson, David Besack, Nyo-nyo Z. Tun, Sara Griffith, Andrew Godwin, and Emmanuelle Nicolas

Fox Chase Cancer Center

at_yeung@fccc.edu

Functional redundancy in Base Excision repair (BER) may permit polymorphism to accumulate in these parallel pathways. Deficiencies in BER may lead to elevated spontaneous mutation rates and an earlier onset of cancer. We have analysed two BER enzymes: TDG and MED1(MBD4), both DNA N-glycosylases that remove the T residue in a T/G mismatch and the U residue in a U/G mismatch. Our analysis of 100 cancer patients revealed only one common polymorphism and no rare polymorphisms for MED1. MED1 polymorphisms are random and appear not to be related to cancer. The TDG gene revealed two common polymorphisms, G199S in exon 5 and V367M in exon 10, and no rare polymorphisms, in our analysis of 268 patients. Curiously, we observed no patient that harbors both polymorphisms when eight such patients were expected based on the allelic frequency of these polymorphisms. If this observation holds true for a larger population study, it may imply that the TDG gene has a second role in humans besides DNA repair, and that the presence of both alleles in a person may lead to embryonic lethality. Such linkage can be useful in evolution to eliminate harmful polymorphisms in important DNA repair genes.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0679 supported this work.